STEM BLIGHT OF ASPARAGUS IN TAIWAN

1. Distribution of the Disease and Cultural Characteristics and Spore Germination of the Causal Organism *Phoma asparagi* Sacc.

Chung-hsiung Hsu and Shou-kung Sun[1]

Abstract

Stem blight of asparagus, incited by *Phoma asparagi* Sacc., was first found in Taiwan by Sawada in 1919. With a rapid increase of the acreage of asparagus since 1963, the disease has been of increasing importance in Taiwan. Survey made in 1968 revealed that an average level of the disease incidence ranged from 24.9% to 66.0% in different asparagus growing areas. Pycnospores primarily invaded the young stem tissue through the base of scale leaves either intact or wounded. Three isolates of the fungus, obtained by single spore isolation, varied in their sporulation on culture media. Pycnidia were abundant with isolate PA-12, moderate with PA-7, and scarce with PA-23, whereas the amount of mycelial growth was more or less in the reverse order. Optimal temperatures for the growth of PA-7, PA-23, and PA-12 were 24°, 32° and 34° C respectively. No growth was observed below 16° C. The best growth of *Phoma asparagi* occurred at pH 7.0. Carbohydrates such as glucose, galactose, xylose, fructose, maltose, saccharose, starch and nitrogenous compounds such as L-cystine, asparagine, L-alanine, $NaNO_3$, etc. can be utilized to different degree by different isolates of the fungus. Conidia germinated well at 28° C with a relative humidity of 96% and failed to germinate at 92%. The optimal pH for germination is 5.6.

Introduction.

Asparagus (*Asparagus officinalis* L.) was first introduced to Taiwan from 1935.[2] During that time Palmetto was the only variety planted. Unfortunately, the cultivation of asparagus was not fully developed because of the Sino-Japanese War (2). Then in 1955 the Taipei District Agricultural Improvement Station introduced three American asparagus varieties, Mary Washington, California 309, and California 711, for trial growing. Satisfactory growth was obtained with all three American varieties as well as Palmetto.

Asparagus for canning was first developed in 1963, with approximately 70 ha. grown at that time. The acreage steadily increased in subsequent years and exceeded 10,000 ha. in 1966. It has become one of the most important canning crops with great economic impact in Taiwan (2,18).

Asparagus has been reported to be attacked by 11 species of fungi, 1 species of bacteria and 1 species of nematodes (1, 4, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17). The stem blight caused by *Phoma asparagi* Sacc. was reported to occur in Taiwan in 1919 by Sawada though of little economic importance (11). At present, the disease becomes one of the most widespread and destructive asparagus diseases in Taiwan. Previous investigations on this disease were confined to its distribution. This series of studies attempt to obtain more information on the disease and the causal fungus as well.

Materials and Methods.

A general survey of some major asparagus growing areas for the disease incidence was made from August to November, 1968. To determine the level of infection, about 0.1 ha. was surveyed for each field and 5-7 fields for each Hsien and municipality were chosen at random. The percentage of disease infection was calculated based on the ratio of the number of diseased stalks to that of healthy ones.

Seeds of Mary Washington were grown in pots in greenhouse. When young stems emerged from sterilized soil, nodes and bud scales on the stem were inoculated either by spraying or by the needle pricking method. For preparation of inocula, conidia were obtained either from pycnidia produced in culture or from diseased tissues. The conidia suspensions were adjusted to the density of 4×10^5 spores per ml. The inoculated plants were kept in dark in a moist chamber at 24°C for 48 hours and then moved to greenhouse at about 20°C.

Single spores were isolated by a glass needle according to Keitt and Langford (7) with some modifications. Three of 33 such single-spore thus obtained were used in various experiments. They were isolates PA-7, PA-12, and PA-23 with moderate, abundant, and scare spore formation on potato sucrose agar.

Media for mycelial growth were prepared from decoctions of potato, carrot, onion, young and old stem or leaves of asparagus. Sporulation and mycelial growth of the three isolates were observed on all those natural media after 5 days at 22±3°C. To determine the effect of pH on mycelial growth, PSA medium was adjusted to various pH values before tubing and, after autoclaving, was transfered to petri dishes, 10 ml per dish. The inoculated plates were incubated at 28° C for 3 days and the diameter of colonies was read.

For determining the ability of utilization by the fungus of different nitrogen or carbon compounds, glucose, galactose, fructose, lactose, maltose, saccharose, starch, mannital, sorbital, inulin, xylose, Na-citrate, Na-oxalate, Na-succinate, Na-acetate, or Na-K-tartarate as carbon source and asparagine, L-alanine, L-cystine, L-histidine HCl, L-theronine, L-valine, $NaNO_2$, or $NaNO_3$ as nitrogen source were incorporated into the

1 Assistant and professor, Department of Plant Pathology, Taiwan Provincial Chung-Hsing University.

2 Asparagus had been cultivated in home gardens before 1935. Commercial variety Palmetto was introduced by 1935 for large scale cultivation.

basic medium of the following composition (mg/1): KH_2PO_4 1000, $K_2HPO_4 \cdot 7H_2O$ 500, $FeSO_4 \cdot 7H_2O$ 7.5, $CaCl_2 \cdot 2H_2O$ 100, $MnSO_4 \cdot 7H_2O$ 2, $CuSO_4 \cdot 5H_2O$ 6, $ZnCl_2$ 75, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 9, biotin 0.005, thiamine 1, and distilled water 1 liter. For the carbon-source media, 3 g/l of KNO_3 and either of the carbohydrates in an equivalent of 8 g of carbon was added into the basal medium separately. For the nitrogen-source media glucose 20 g/1 and an equivalent of nitrogen 415.4mg (=KNO_3 3g) from the different sources were added. The carbon compounds, nitrogenous compounds, biotin, and thiamine were added to previously sterilized basic medium and further autoclaved for 10 min. at 10 lbs per sq. in.. To each 50 ml Erlenmeyer flask, 25 ml of test medium was dispensed. Mycelial mats, one ml, from 7-day-old cultures in Czapek's solution ($NaNO_3$ 3g, K_2HPO_4 1g, $MgSO_4 \cdot 7H_2O$ 0.5g, KCl 0.5g, $FeSO_4 \cdot 7H_2O$ 0.01g, sucrose 30g, and distilled water 1,000 ml), were inoculated into these liquid media. The inoculated flasks were incubated by constant shaking at 28°C for 11 days. The mycelial mats were washed, filtered, dried in an oven at 55°C for 24 hr. and weighed.

Spore germination was tested under different temperatures, pH values, and relative humidity. Spore suspension prepared with well-water (pH 6.5) was placed on glass slides coated with glycerin. The slides were placed in petri dishes and incubated at different temperatures for 48 hr. The moisture in petri dishes was maintained by moist filter papers. Buffered solutions for suspending the spores and testing their germinability at pH 4.5—8.0 were prepared by mixing different amounts of 0.1M Na_2HPO_4 and 0.1M KH_2PO_4, while a pH value in the range of 1.8–4 and of 9.0 were obtained by adjusting distilled water with 0.1 N HCl or 0.1 N NaOH. Spore were germinated in buffer or distilled water of a given pH on the glycerin-coated slides and kept in an incubator at 28°C for 48 hr. Moisture chambers with various levels of relative humidity were made with saturated salt solutions according to Clayton (3). Conidia were suspended in egg-white over a slide while they were incubated at 28°C for 48 hours at different humidity levels. Spores were killed by 0.1% $HgCl_2$ solution at the end of incubation period and examined.

Results.

(1) *Distribution of the disease*: Survey on the distribution of the stem blight disease made from August to November in 1968 revealed that the disease was widespread in Taiwan but severity of infection was not related to latitudes. Fig. 1 summarizes the results.

The highest infection of the disease was 90 percent as observed in the vicinity of Pingtung Hsien, while the lowest was 16 percent in Tung-hsiao of Miaoli Hsien.

(2) *Symptoms*: The disease attacks the aerial parts of the plant. The first evidence of the disease is the appearance of minute discolored areas on the node around the scale leaves. With the progress of the disease, tiny elliptical spots with water-soaked margin are evident. The lesions soon enlarge to about 2×5 mm or more with central parts becoming light greenish-brown in color. A typical lesion, usually 0.2-0.5×2.0-3.0

cm in size, is on the cast of gray to ashy-white with dark purplish brown margin. The central tissue of the spots becomes sunken and dotted with white structures which are the initial stage of the pycnidia of the causal organism. As the tissue dries out, these minute dots turn brown, dark-brown or black. Sometimes the lesions coalesce and longitudinal cracks may appear on the stem. If attacked in their early stage of growth, the lateral branches may dry up and die. Growth is consquently arrested on all parts of the plant when large lesions appear on matured or near-matured stems. Stalks may be killed when infection occurs soon after their emergence from soil surface. In serious cases the needles turn yellow, and partial or complete desiccation will ensue (Fig. 2).

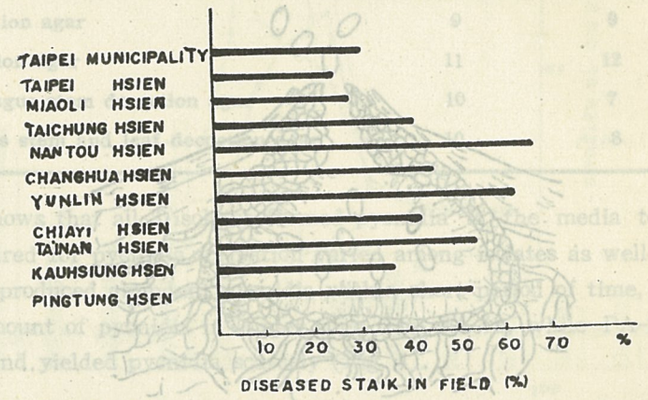

Fig. 1. Level of disease incidence of stem blight in some of the asparagus growing areas in Taiwan.

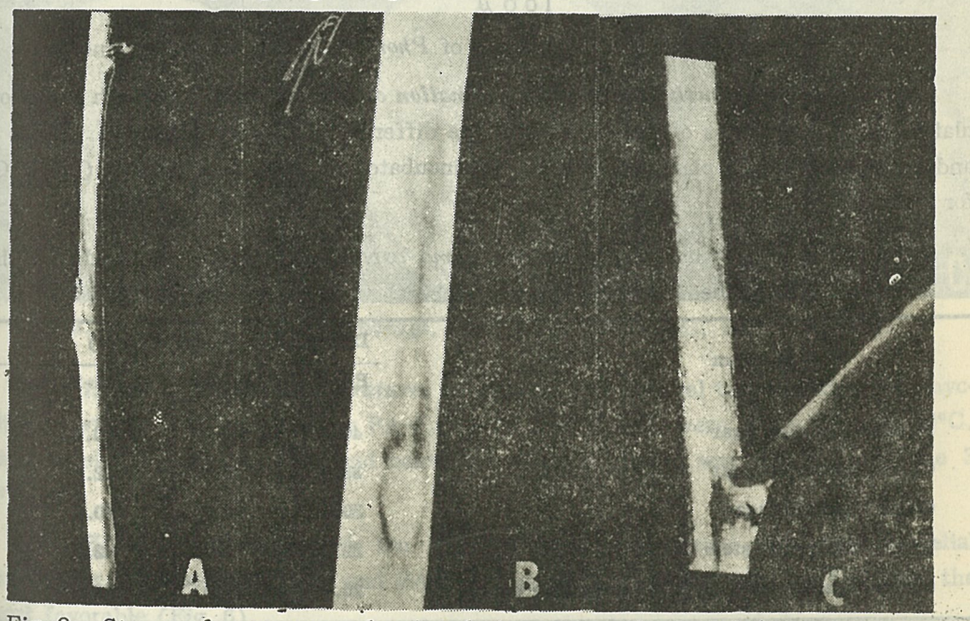

Fig. 2. Stems of asparagus showing lesions on scale leaf (A), lesions with water-soaked margin on stem (B), and pycnidia on old stem lesion (C).

(3). ***The Causal organism***: ***Phoma asparagi*** was first described by Saccardo in 1884 (10). Dark brown pycnidia are embedded in the diseased tissues of the stems or branches. They are elliptical to flask-shaped, with beaked or papillate ostioles when mature. The size of pycnidia measures 78–200×65–154 μ (average 132×94 μ). The pycnidial wall is 13–45 μ thick. The conidiophores, 14–22×3.5–6.0 μ, arise from the inner wall of pycnidia, standing together, hyaline, pointing toward the center of the pycnidia. The conidia are hyaline, non-septate, spherical or oval, 5.0–12.9×2.5–5.6 μ (Fig. 3). The conidia are produced in abundance within the pycnidium. Hyphae are septate, branched, hyaline when young, becoming dark-walled with age on artificial media.

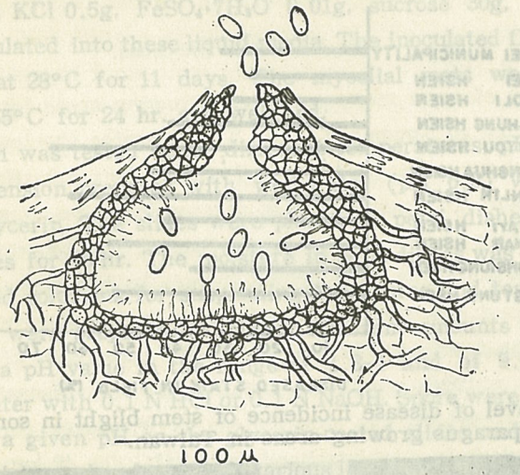

Fig. 3. Cross section of Pycnidium of ***Phoma asparagi*** in host tissue.

(4). ***Vegetative growth and pycnidia formation on various media***: Growth and sporulation of the 3 isolates of the fungus in five different media are shown in Tables 1 and 2. In each case 4 plates were used and incubated at room temperature (22±3° C) for 5 days.

Table 1. The growth of 3 isolates of ***Phoma asparagi*** on different kinds of the natural media after 5 days at 22±3°C

Medium	Diameter of colonies (mm)		
	PA-7	PA-12	PA-23
Potato decoction agar	40.0	42.5	60.0
Carrot decoction agar	38.8	40.0	42.0
Onion decoctionagar	30.5	24.3	60.0
Young asparagus stem decoction agar	32.8	35.5	42.5
Old asparagus stem and leaf decoction agar	26.6	23.8	32.5

The mycelial growth of PA-23 was the fastest of the 3 isolates. For all 3 isolates the potato decoction was the most favorable, while the decoction of young asparagus stems was superior to that of old asparagus stems and leaves.

Table 2. Days required for pycnidia formation on various media of the 3 isolates of ***Phoma asparagi***

Medium	Days required for pycnidia formation		
	PA-7	PA-12	PA-23
Potato decoction agar	9	7	12
Carrot decoction agar	9	9	8
Onion decoction agar	11	12	11
Young asparagus stem decoction agar	10	7	9
Old asparagus stem and leaf decoction agar	10	8	18

Table 2 shows that all 3 isolates formed pycnidia in the media tested. However, the time required for pycnidia formation varied among isolates as well as on different media. PA-12 produced abundant pycnidia within short period of time, PA-7 produced a moderate amount of pycnidia in longer time than PA-12, while PA-23 required the longest time and yielded pycnidia scarcely (Fig. 4).

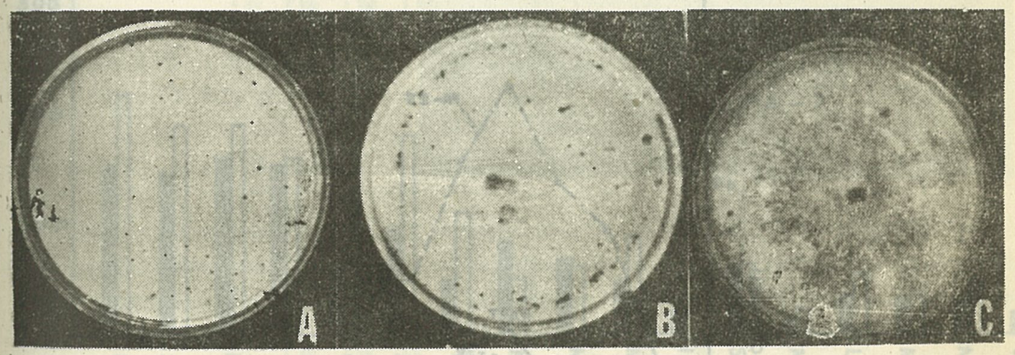

Fig. 4. Isolates of ***Phoma asparagi***, PA-7 (A), PA-12 (B), and PA-23 (C).

(5). ***Effect of various temperatures on growth***: The optimal temperatures for mycelial growth on PSA medium for PA-7, PA-12 and PA-23 were 24°, 34° and 30°C, respectively (Fig. 5). At 16°C and below, no mycelial growth was observed for the 3 isolates.

(6). ***Effect of pH on growth***: On PSA adjusted to different pHs, best mycelial growth for both isolates PA-23 and PA-7 was at pH 7.0. For PA-12, pH 8 was the most favorable (Fig. 6).

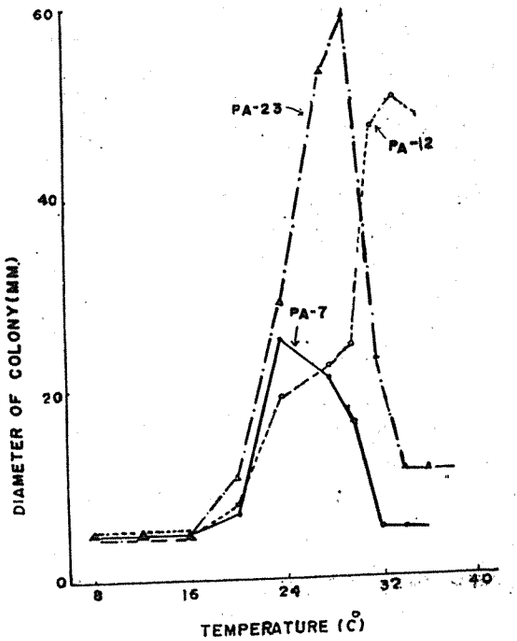

Fig. 5. Effect of temperatures on mycelial growth of 3 isolates of *Phoma asparagi*.

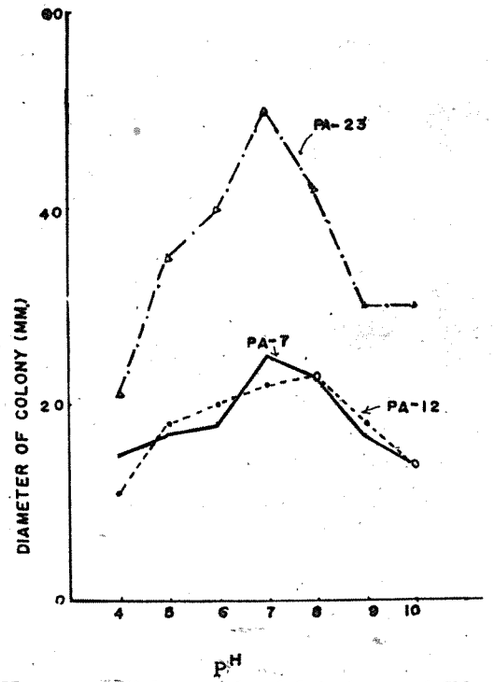

Fig. 6. Effect of pH on mycelial growth of 3 isolates of *Phoma asparagi* at 28°C.

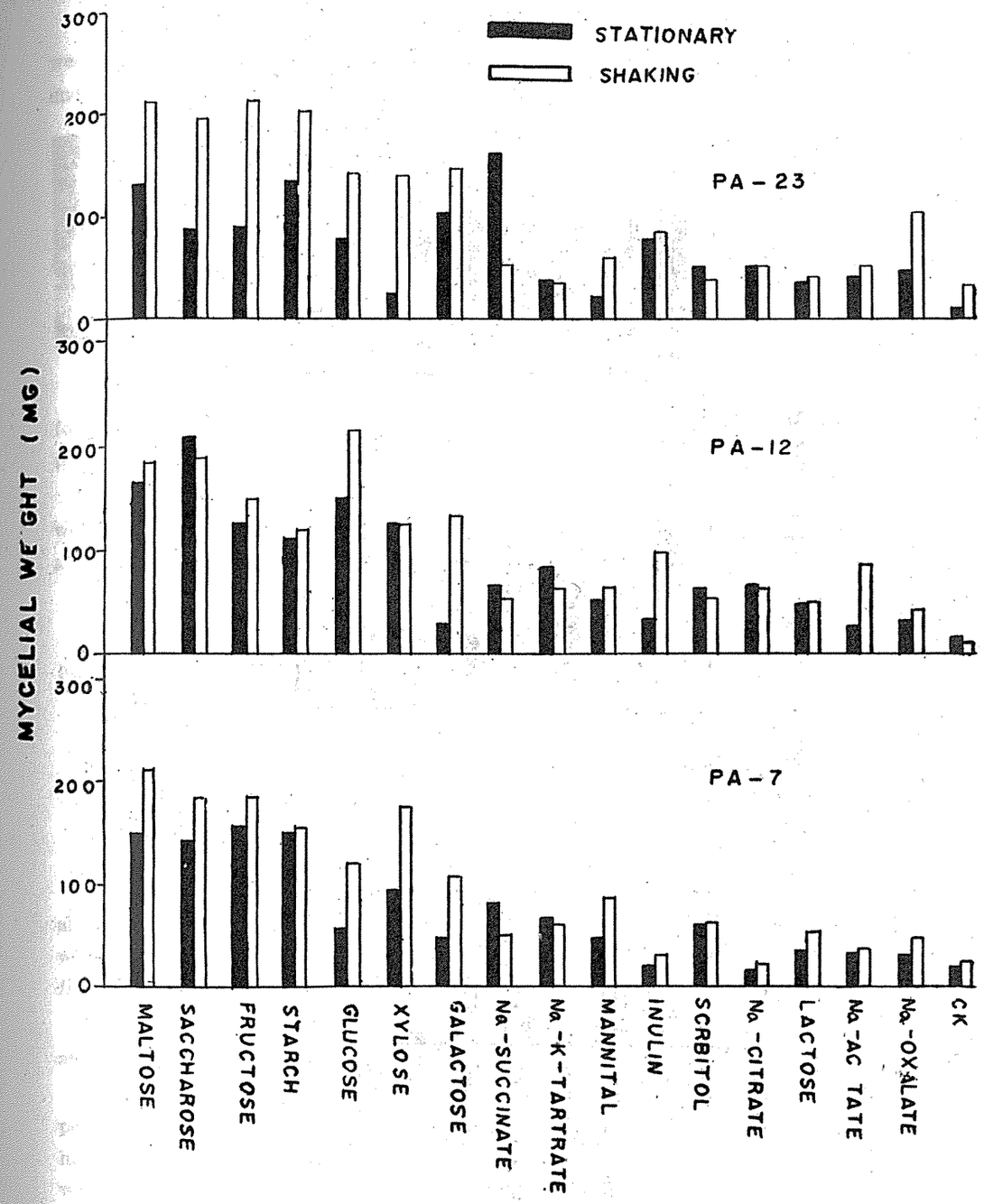

Fig. 7. The ability of 3 isolates of *Phoma asparagi* to use carbon sources as determined by the oven-dry weight of mycelial mats.

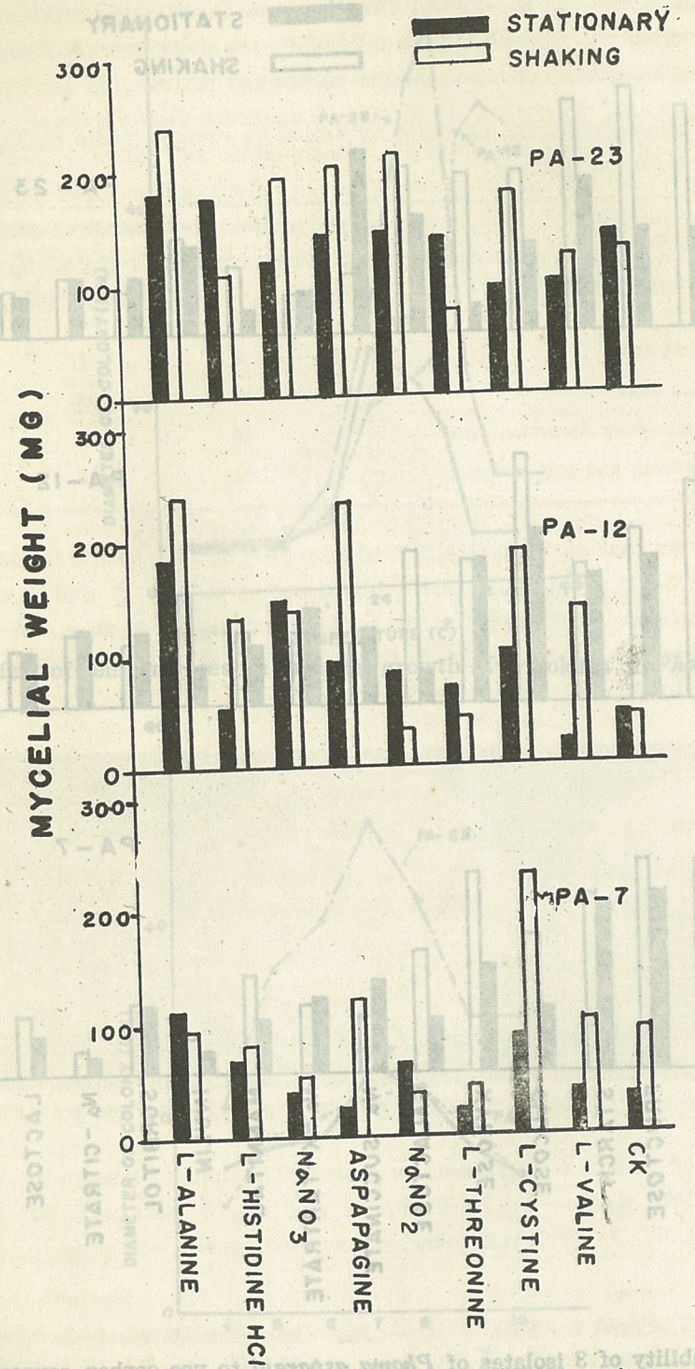

Fig. 9. The ability of 3 isolates of *Phoma asparagi* to use nitrogen sources as determined by the oven-dry weight of mycelial mats.

(7). ***Effects of carbohydrates on mycelial growth***: Sixteen kinds of carbohydrates were used as carbon-source for mycelial growth of the fungus. The results of stationary and shaking cultures are shown in Figs. 7 and 8.

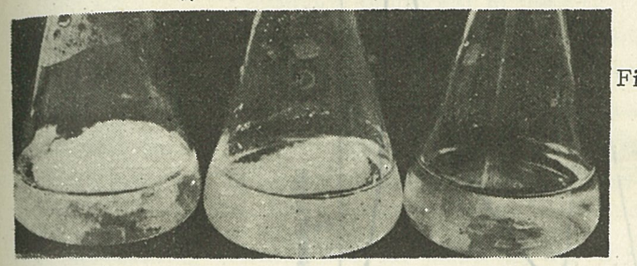

Fig. 8. Growth of isolate PA-12 on glucose (left), lactose (middle), and basic medium (as ck).

Glucose, galactose, xylose, fructose, maltose, saccharose, and starch were satisfactory for mycelial growth of the 3 isolates. Na-succinate was good for PA-23 and Na-K-tartrate for PA-12 only.

(8). ***Effect of nitrogens on mycelial growth***: Eight kinds of nitrogenous compounds were used as nitrogen-source on mycelial growth of the fungus. The results of stationary and shaking cultures are shown in Figs. 9 and 10.

Fig.10. Growth of *Phoma asparagi* on $NaNO_2$ medium for PA-7(left), PA-12(middle), and PA-23(right).

The L-cystine was the best for mycelial growth of isolate PA-7. Asparagine, L-alanine, L-cystine, and $NaNO_3$ were more satisfactory for PA-12 than other nitrogens, while asparagine, L-alanine, L-cystine, L-histidine HCl, $NaNO_3$ and $NaNO_2$ were more favorable for PA-23.

(9). ***Effect of temperatures on conidia germination***: The results of conidia germination at different temperatures are shown in Fig. 11.

The optimal temperature for the conidia germination was 28°C at which 88.4 percent of spores germinated. Conidia failed to germinate at lower (4 and 8°C) and higher (38° C) temperatures. But their germinability remained and germination resumed when they were moved to the optimum temperature (28° C) for germination. Nevertheless, incubation at 38°C for 8 days irreversibly destroyed the spore germinability. The data are shown in Table 3.

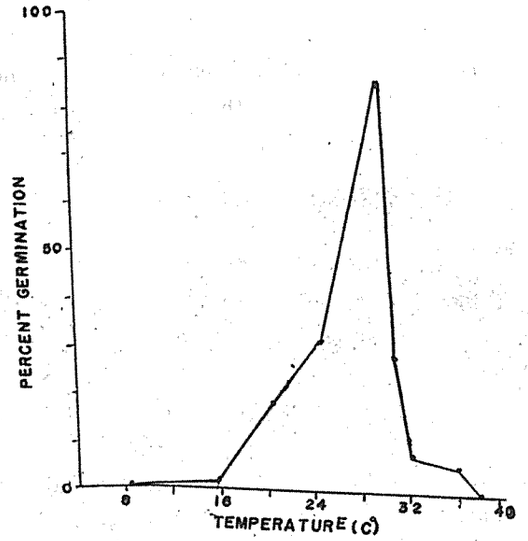

Fig. 11. Effect of temperature on germination of *Phoma asparagi*.

Table 3. Survival of conidia after exposing to unfavorable temperatures and then removing to 28°C

Incubation time at unfavorable temperatures (days)	Incubation time at 28°C (days)	Percent germination after exposing to		
		4°C	8°C	38°C
8 (Control)	0	0	0	0
1	1½	28	39.9	5.7
8	2	28.2	40.9	0

(10). ***Effect of pH on conidia germination***: The results of different pH values on conidia germination are shown in Fig. 12. At pH 5.9, about 90 per cent of germination was obtained.

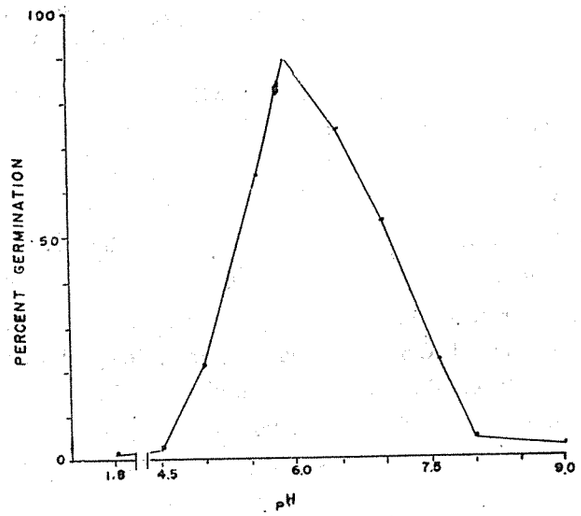

Fig. 12. Effect of pH on the germination of conidia of *Phoma asparagi*.

(11). ***Effect of relative humidity to conidia germination***: The results of different relative humidities on conidia germination are shown in Figs. 13 and 14.

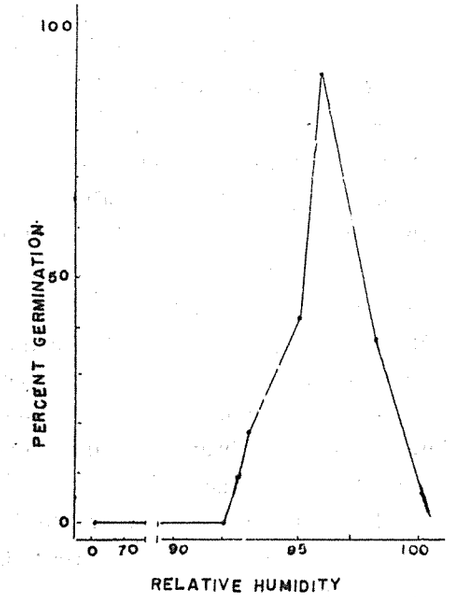

Fig. 13. Germination of conidia of *Phoma asparagi* at different relative humidities.

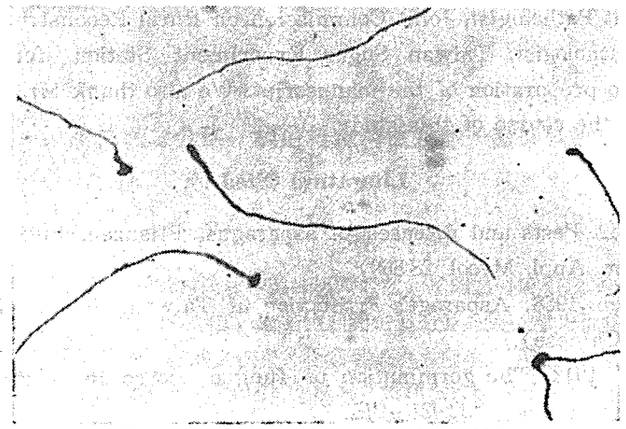

Fig. 14. Germination of conidia.

Conidia germinated within the humidity range of 93~100%. Germination was best at 96%, fair at 100%, and failed to occur at 92% of relative humidity and below.

Discussion

The stem blight disease of asparagus is widespread and destructive in Taiwan. The disease not only reduces the vigor of plants but also causes the death of the stalks when young plants are infected.

Lesions on the plant naturally infected are confined to bud scales and expand gradually toward the upper parts of the scale leaf. This could be verified by artificial inoculation. In these cases, young tissues and bud scales provided sites of successful infections and for subsequent development of the causal organism.

Germination of conidia *in vitro* was favored by a low pH. Measurment on soil samples from some of the asparagus fields indicated that the disease incidence was high in the fields with a lower soil pH. Since mycelial growth was favored by pH 7, it remains to be determined whether the factor affecting conidia germination plays much more important role than that affecting mycelial growth in the epidemics of the disease.

Since temperatures above 20°C are favorable for both conidia germination and mycelial growth of the fungus, it is no wonder that cold weather reduces the severity of the disease in the winter season in Taiwan. Furthermore, conidia require a mininum relative humidity of 93% at 28°C for germination, a condition not present in most asparagus fields in the winter season in Taiwan. This also implicates that the disease is likely to increase its severity in the summer season in Taiwan.

Further studies on the epidemics of the disease are to be focused on whether temperature plays much more important role to affect disease incidence than the pH value of soil in the field of asparagus.

Acknowledgement: The writers wish to acknowledge their indebtedness to Dr. R. J. Chiu, Senicr Plant Pathologist, Joint Commission on Rural Reconstruction and Dr. L. S. Leu, Plant Pathologist, Taiwan Sugar Experiment Station, for their valuable suggestions in the preparation of the manuscript. We also thank Mr. C. C. Yeh for his assistance during the course of this study.

Literature Cited

1. Bollow, H. 1949. Pests and diseases of asparagus. Pflanzenschutx, 1(9): 105~108. (Abst. in Rev. Appl. Mycol. 28:560)
2. Chen, Chiu-Jen. 1968. Asparagus Production in Taiwan. Taiwan Bank Bul. 19:1, 209~244 (In Chinese).
3. Clayton, C. N. 1942. The germination of fungus spores in relation to controlled humidity. Phytopathology 32:921~943.
4. Cook, M. T. 1923. Dwarf asparagus. Phytopathology 13: 284. (Abst.)
5. Graham, K. M. 1952. A fusarial disease of seedling asparagus in Ontario. Phytopathology 42:9 (Abst.)
6. Kahn, Robert P., H. W. Anderson, and Paul R. Hepler. 1952. Asparagus rust investigation in Illinois. Phytopathology 42:13. (Abst.)
7. Keitt, G. W. and M. H. Langford. 1941. *Venturia inaequalis* (Cke.) Wint. I. A ground work for genetic studies. Amer. J. Bot. 28:805~820.
8. Kheswalla, K. F. 1937. A phoma disease of asparagus. Indian J. Agr. Sci. 6(3): 800~802
9. Ogilvie, L. 1930. Observations on the diseases of market-garden crops in the vale of Evesham. Ann. Rep. and Hort. Res. Sta., Long Ashton, Bristol, for 1929, pp. 149~154. (Abst. in Rev. Appl. Mycol. 9:700)
10. Saccardo, P. A. 1884. Sylloge Fungorum III. p. 162.
11. Sawada, K. 1919. Descriptive catalogue of Fungi found in Taiwan. (In Japanese) Vol. 1, Taiwan Agr. Res. Inst.
12. Siemaszko, W. 1925. Phytopathological Notes 1. Chorobyi Szkodniki, Roslin (Plant Disease and Pests) 1, 4pp. (Abst. in Rev. Appl. Mycol. 4:380)
13. Smith, E. H. 1924. Some diseases new to California. Phytopathology 14:125. (Abst.)
14. Sydow, H. and W. McRae. 1930. Hyphomycetes Indiae Orientalis Pars I. (East Indian Hyphomycetes. Part I.) Ann. Cryptog. Exot., ii 3~4pp. 262~271. (Abst. in Rev. Appl. Mycol. 9:612~613).
15. Tehon, L. R. and G. L. Stout. 1930. Notes on the parasitic fungi of Illinois. Mycologia 21(4): 62~196.
16. Teng, S. C. 1933. Fungi of Nanking II. Contrib. Biol. Lab, Sci, Soc, China Bot. Ser., 8(1): 5~48. (Abst. in Rev. Appl. Mycol. 12:395~396)
17. Walker, J. C. 1952. Diseases of vegetable crops. pp. 1~9. McGraw-Hill Co., New York, U. S. A.
18. Department of Agriculture and Forestry, Provincial Government of Taiwan 1968. Taiwan Agricultural Yearbook (for 1967)

蘆 筍 莖 枯 病 之 研 究

一、莖枯病之分佈，病菌培養特性及胞子發芽試驗

許 忠 雄　孫 守 恭

中 文 摘 要

蘆筍莖枯病早在民國八年澤田兼吉氏報告其在臺灣發生，然因當時蘆筍栽培面積少，此病未見嚴重，亦未有進一步之研究。自民國52年起，蘆筍罐頭開始製造並外銷，栽培面積大增，莖枯病亦隨之普遍而猖獗。據民國57年8～11月間之調查，全省各地均有發生，平均罹病率自24.9%至66.0%。

莖枯病病原菌（*Phoma asparagi* Sacc.）之柄胞子可經傷口或非傷口侵入幼莖，尤多自節間之鱗片處侵入。單胞分離選出三個菌株，此三菌株在天然培養基上，其柄子器形成之多少甚有差異，PA-12 菌株形成柄子器最多，PA-7 菌株居中，PA-23 菌株最少，而菌絲生長略與柄子器之形成相反。PA-7，PA-23及PA-12之生長適溫各爲24°，30° 及34°C，溫度在16°C則無生長。本菌生長最適 pH 爲7.0或略高於7。

蘆筍莖枯病菌可利用多種炭水化物，如葡萄糖，半乳糖，木質糖，果糖，麥芽糖，蔗糖及澱粉，但菌株間利用能力有差異。本菌亦可利用氮素源化合物如重胱氨基酸（L-cystine），天冬素（Asparagine），丙氨酸（L-alanine），硝酸鈉等。當溫度爲28°C時，胞子在相對濕度96%時發芽最好，相對濕度92%時即停止發芽，發芽最適氫離子濃度爲pH 5.6。